Ecological Indicators 85 (2018) 318–328

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Research paper

Fatty acids profiles modifications in the bivalves *Cerastoderma edule* and *Scrobicularia plana* in response to copper sulphate

A.F. Mesquita[a], F. Gonçalves[a], T. Verdelhos[b], J.C. Marques[b], A.M.M. Gonçalves[a,b,*]

[a] Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[b] IMAR (Marine and Environmental Research Centre) & MARE (Marine and Environmental Sciences Centre), Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal

ARTICLE INFO

Keywords:
Cerastoderma edule
Scrobicularia plana
Fatty acids
Toxicity
Bioindicators
Copper sulphate

ABSTRACT

At the past 30 years were recorded an intensive practice in the use of fertilizers and pesticides, mainly in the European Mediterranean region, that, in particular cases, exceeded the limits of regular legislations established by the European Union. The widespread use of these chemicals compounds and the pressure over agricultural fields near valuable ecologically coastal areas conducted to the implementation of monitoring plans to the recovering of aquatic ecosystems. Since the 80's Mondego estuary (Figueira da Foz, Portugal) has been harassed by anthropogenic pressures, which triggered the implementation of mitigation monitoring programs at the last 18 years, allowing its recovery. Copper sulphate is used in industrial activities, but also it is much used in pesticides formulations, with application in agricultural activities, namely in rice farms to control pests. Studies reported that copper may affect biochemical processes, such lipid metabolism of some organisms, although specific changes in fatty acid (FA) profiles are still unknown. Nowadays, bivalve species are used in ecotoxicological bioassays due some particular characteristics, such as the wide distribution, ecological relevance, the capacity to filter and ingest large volumes of sediment particles and water and ease handling in the field and in the laboratory. Therefore, this work aims to determine toxic effects and changes in fatty acids profile composition of the two marine bivalve species *Cerastoderma edule* and *Scrobicularia plana* when exposed to copper sulphate, considering small (medium body size = 1.97 cm and 3.47 cm, respectively) and big (medium body size = 2.45 cm and 4.20 cm, respectively) size classes. In a first phase organisms were exposed under laboratorial conditions to copper sulphate to determine lethal concentration; at a second phase, it was compared the FA profile and the nutritive quality of both species and size classes at the field and in the lab. Our results state *C. edule* is more sensitive to copper sulphate (LC_{50} = 0.818 (0.595–0.987) mg/L; 1.129 (0.968–1.289) mg/L, to big and small organisms, respectively) than *S. plana* (LC_{50} = 2.563 (2.229–2.903) mg/L; 4.705 (3.540–12.292) mg/L, to big and small organisms, respectively). Furthermore the last one presents greater abundance and variety of FA and essential fatty acids (EFA), namely DHA and EPA, rates than *C. edule*. Still, big size class of both bivalve species is the most affected by the contaminant.

1. Introduction

Estuaries are highly subjected to metals (e.g., Cd, Cr, Pb, Hg, Ni, Cu) that are consider an important group of pollutants in these systems affecting the physiological and biochemical integrity of aquatic organisms. These stressors may be from natural (volcanic activity, vegetation decrease) or anthropogenic sources (industrial processes, agricultural activities or use of antifouling paints). At the last decades, the production of copper has increased and consequently also the pollution by this metal. Moreover, in some cases, it achieved high concentrations, mainly in agriculture fields due to an overuse of pollutants where copper is one of the main constituents (Parry and Pipe, 2004; Lencioni et al., 2016).

Copper sulphate is used on industrial activities, but also in pesticides formulations (as herbicide, fungicide, molluscide or algicide), with application in agricultural activities, namely in rice farms to control pests like *Isidorella newcombi* (Stevens et al., 2014). Copper is an essential element to the normal performance of the organisms, acting as cofactor of many enzymes, i.e., it is a component of superoxide dismutase, an enzyme defending living organisms against reactive oxygen species (Barman, 1974), however in high concentrations or chronic exposure to low concentration, it may become toxic. Copper can cause

* Corresponding author at: Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal/IMAR (Marine and Environmental Research Centre) & MARE (Marine and Environmental Sciences Centre), Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal.
E-mail addresses: anamartagoncalves@ua.pt, anamartagoncalves@gmail.com (A.M.M. Gonçalves).

http://dx.doi.org/10.1016/j.ecolind.2017.10.007
Received 29 July 2017; Received in revised form 2 October 2017; Accepted 4 October 2017

the commitment of reproduction and growth rates, feeding mechanisms, and also the increase and development of histopathological anomalies and sickness (Al-Subiai et al., 2011). Moreover, copper may cause several biochemical effects (Viarengo, 1985; Gaetke and Chow, 2003; Filimonova et al., 2016a, 2016b; Lencioni et al., 2016), such as the transition metals, that catalyses the production of reactive oxygen species (ROS) causing oxidative stress on several biomolecules (Sies, 1986).

Therefore, it is necessary and highly relevant to investigate the influence of these pollutants on non-target species. The application of biomarkers to determine the effects of different stressors on biochemical pathways that regulate the organism's health and fitness will provide higher detailed information than indirect measurements, being used as sensitive early warning bio-indicator of stress (Vieira et al., 2009; Gonçalves et al., 2012, 2016; Neves et al., 2015; Filimonova et al., 2016a, 2016b).

Fatty acids molecules are important compounds due to its presence in neural system and at several biochemical pathways, being a source of energy and one of the main constituents of cell membranes developing several functions at the activity of membrane proteins and signals, at the traffic of cell compounds and on the action of membrane permeability (Ibarguren and López, 2014; Liu et al., 2015). Polyunsaturated fatty acids (PUFA), where are included some of the essential fatty acids (EFA) and some omega 6 and omega 3, are a family of lipids that contains some subgroups identified by the position of the last double bond in their structure. The terms "PUFA" and "EFA" are not synonymous, although they are always used indistinctly as most of the biological functions of EFAs are exerted by EFA–derived PUFAs. Animals may convert PUFA by desaturation or elongation, still a few are able to synthetize this group of molecules, with the ratio between the amount produced and the energy spent not worthwhile, with animals obtaining PUFA mainly by food sources (Brett and Müller-Navarra 1997). This group of FA serves as precursors of important hormones and are essential to the regulation of cell membranes (Neves et al., 2015). Highly unsaturated fatty acids (HUFA) like eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are related to reproductive and growth rates success, neural development, presenting a key role at the functional and health status of all animals from all trophic levels. HUFA are essential metabolites that cannot be synthesized de novo, or at least not in sufficient quantities, being taken up via food sources (Ladhar et al., 2014). HUFA are nutritional key constituents of bivalves diet and are the algae nutritional value for bivalves, determined by EFA (Hendriks et al., 2003).

Although the consumption of nutrients by bivalves become from many material particles, like resuspended benthic microalgae, phytoplankton, detritus from many sources, bacterial and microheterotrophos, they are classified as herbivorous with phytoplankton being their primary food source (Pernet et al., 2012). At this work two important commercial bivalve species, *Cerastoderma edule* and *Scrobicularia plana,* were investigated.

Cerastoderma edule presents a widely geographical distribution, from North Africa to Northern Norway and Murmansk in the Arctic, present also on the east coast of the Atlantic (Freitas et al., 2014). It is a suspension-feeder living on intertidal shallow areas, with an important role between primary producers and consumers (Verdelhos et al., 2015). Due to the large filtration ability of *C. edule* to accumulate high quantities of pollutants this species is widely applied as bioindicator in ecological studies (Paul-Pont et al., 2010a, 2010b; Nilin et al., 2012; Cardoso et al., 2013; Freitas et al., 2014). *Scrobicularia plana* also shows a wide distribution from Norway to the Mediterranean and West Africa. *S. plana* is a deposit filter feeder, inhabiting intertidal and subtidal areas, burrowing on mud to muddy sand sediments to a depth of 25 cm (Verdelhos et al., 2015). This species has a great ability to accumulate and filtrate pollutants that accumulates in the digestive gland (Paul-Pont et al., 2010a, 2010b).

Bivalves are considered standard species in ecotoxicology due to their sessile life style, easy sampling collection, maintenance, handling and sensitivity to chemicals (Gonçalves et al., 2016). The molecular and physiological responses of these species to organic and inorganic compounds may be used as earlier indicators of potential ecosystem damages (Nilin et al., 2012). Moreover, the relevance of these organisms is also related to their socio-economic importance, since they are extremely appreciated as food source. By all this, ecotoxicological studies are crucial to determine and assess the effects of contaminants in aquatic communities and thus to determine the most suitable indicators to be used as early warning-signals. Thus, in the present study are investigated: 1) the toxic effects of copper sulphate on *Cerastoderma edule* and *Scrobicularia plana*, 2) the effects on FA profiles of *C. edule* and *S. plana* when exposed to the contaminant and 3) the changes on the nutritious quality of both marine bivalves species, and size classes, at the field and after the exposure to the toxic under laboratory conditions.

2. Materials and methods

2.1. Study site and field sampling

The Mondego estuary is located in a Mediterranean region, on the Atlantic coast of Portugal (40°08′N, 8°50′W), near Figueira da Foz city, Portugal (Fig. 1). It is a small estuary extended for about 8 km and covers an area of approximately 3.4 km^2. The estuary contains two arms, north and south, separated by the Murraceira Island. The

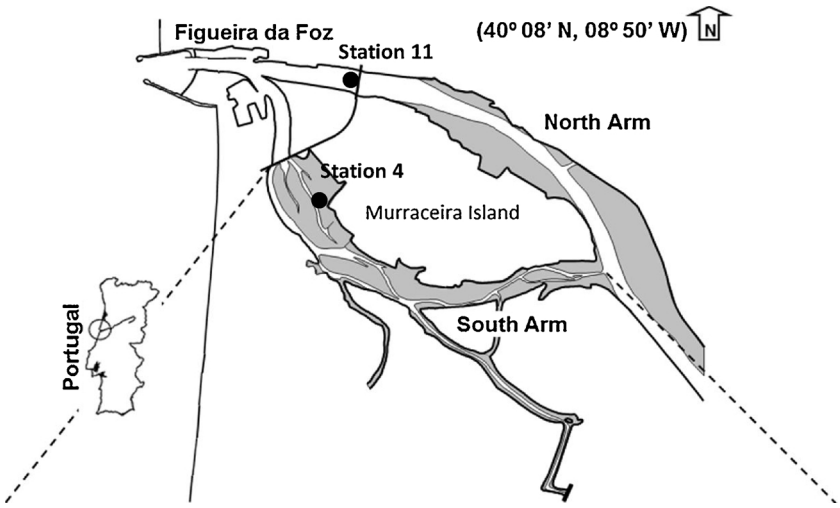

Fig. 1. Location of the Mondego estuary and the sampling sites within the estuary.